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(54) Title: A METHOD OF DETECTING HTLV-I ANTIBODIES IN HUMAN BODY FLUIDS

(57) Abstract

The present invention relates to a HTLV-I immunoassay which uses recombinant antigenic peptides encoded by the *env*, *tax* and *gag* genes of the HTLV-I virus. The use of a combination of these antigenic polypeptides provides a screening assay for detecting the presence of HTLV-I antibodies in body fluids by combining body fluids containing antibodies with one or more recombinant polypeptide antigens selected from the group consisting of polypeptides encoded by all or part of the *env*, *tax* and *gag* genes of HTLV-I, forming a detectable antibody-antigen binding pair and detecting the presence of the binding pair.

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A METHOD OF DETECTING HTLV-I ANTIBODIES IN HUMAN BODY FLUIDS

The present invention relates generally to immunoassays for the detection of HTLV-I antibodies in body fluids and, more particularly, to highly accurate and reliable HTLV-I screening immunoassays based on the use of multiple HTLV-I recombinant antigenic polypeptides.

BACKGROUND OF THE INVENTION

Human T-Cell Leukemia Virus, HTLV-I, is a retrovirus etiologically associated with adult T-cell leukemia and tropical spastic paraparesis. HTLV-I infection is endemic in some parts of the world, including southern Japan, Okinawa, and the Caribbean basin. The prevalence of infection in these endemic areas is about 5 to 10% and may be higher in specific locations.

Based upon blood donor studies, the prevalence of HTLV-I infection in the United States is now about 0.025%. This level of seropositive blood donors raises concerns for possible transmission of HTLV-I by exposure to contaminated blood products.

Individuals infected by or exposed to HTLV-I mount an immune response to viral proteins by generating antibodies to these proteins. Some of the HTLV-I proteins which are or may be immunogenic are encoded by the gag, env and tax genes of the HTLV-I genome. The immunogenic proteins encoded by these genes are potentially important antigenic materials for configuring a sensitive blood screening assay to detect HTLV-I antibodies in blood samples. See, generally, Slamon, et al., Science 228:1427-1430 (1985), and Lee, et al., Proc.Natl.Acad.Sci.USA., 81:3856-3860 (1984).

Currently, Food and Drug Administration approved blood screening assays are available which may be used to detect the presence of HTLV-I antibodies in blood samples. Available screening assays are discussed in Morbidity and Mortality Weekly Report, 37, No. 48, 737-747 (1988). These assays typically employ viral antigenic proteins isolated from mammalian cell cultures which are infected with HTLV-I. See, generally, Sawada, et al., U.S. Patent No. 4,588,681; Essex, et al., PCT Application WO 84/04327, published November 8, 1984; Copeland, et al., J. of Immunol., 137:2945-2951 (1986); Saxinger, et al., Laboratory Investigation, 49:371-377 (1983); Bodner, et al., EPA 0136798, published April 10, 1985; Tsuji, et al., EPA 0135352, published March 27, 1985. It is known, however, that the recovery of certain viral proteins from HTLV-I infected mammalian cells is generally poor and that other non-viral mammalian cell proteins may contaminate the recovered viral proteins. Thus, the isolation of significant quantities of highly purified HTLV-I proteins from infected mammalian cells may be impractical and contaminating mammalian proteins may lead to unreliable assay results. Assays which use the live virus as part of the manufacturing process also create a safety hazard for workers because they may be exposed to infected cells or infected cell products when assembling the assay. The development of a practical, specific, and sensitive assay for HTLV-I is thus burdened because of the difficulties of working with infected cells and infected cell products.

Some of the problems associated with use of HTLV-I proteins derived from infected mammalian cells may be overcome by applying recombinant DNA methods and techniques to develop antigenic polypeptides in non-mammalian host cells. Unfortunately, the direct expression of HTLV-I derived polypeptides in heterologous cell systems has been problematic. See,

generally, Samuel, et al., Science, 226:1094-1097 (1984); Giam, et al., Proc.Natl.Acad.Sci.USA., 83:7192-7196 (1986); Jeang, et al., J. of Virol., 61:708-713 (1987); and Papas, et al., U.S. Patent Application Serial No. 06/664,972 filed October 26, 1984, available from the National Technical Information Service, Springfield, Virginia. The expression of antigenic polypeptides based on use of all or portions of the proviral DNA sequence has not been particularly successful. Researchers have resorted to alternative methods for making these polypeptides, such as expressing HTLV-I antigenic polypeptides as fusion products or transforming heterologous cells with composite synthetic/native DNA sequences which encode all or portions of HTLV-I proteins. For examples of fusion polypeptide products see, generally, Itamura, et al., Gene, 38:57-64 (1985); Sanchez, et al., Virology, 161:555-560 (1987); Slamon, et al., PCT Application W086/01834, published March 27, 1986; Itoh, et al., U.S. Patent 4,795,805; Yoshida, et al., U.S. Patent 4,794,258; Yoshida, et al., EPA 0 151 475, published August 14, 1985; Sugano, et al., EPA 0 152 030, published August 21, 1985; and Taniguchi, et al., EPA 0 139 216, published May 2, 1985.

HTLV-I assays employing recombinant antigenic polypeptides have been described. For example, antigenic polypeptides expressed in E. coli transformed with portions of the gag gene may be used in an immunodot assay. The sensitivity of this immunodot assay was described as being comparable to Western blots and the results were described as being as reliable as radioimmunoassays, Kanner, et al., J. Immunol. 137:674-678 (1986). For another assay employing antigenic polypeptides encoded by the gag gene, see Itoh, et al., U.S. Patent 4,795,805.

Other HTLV-I derived recombinant antigenic polypeptides have also been used in immunoassays. Cell lysates containing either a 59 kD fusion polypeptide encoded by about half of the env gene and about three-quarters of the tax gene or a single 100 kD fusion polypeptide encoded by gag, env and tax gene fragments reacted with sera from an HTLV-I infected patient using a Western blot analysis, Kitajima, et al., Molecular and Cellular Probes, 2:39-46 (1988).

A sensitive HTLV-I assay which employs recombinant antigenic polypeptides requires antigens which are readily available and which are immunologically reactive with antibodies found in all or nearly all seropositive individuals. These antigenic polypeptides must be readily purified in order to avoid or eliminate non-specific binding to contaminating host cells proteins by cross-reactive antibodies which may be present in body fluid samples. These antigenic polypeptides must also retain their immunological activity when they are used to prepare immunoassay apparatus which typically involve adsorption of the antigenic polypeptides onto a solid support and contacting the adsorbed polypeptides with various blocking and washing reagents.

In addition, a sensitive HTLV-I assay requires more than one antigen in a single assay. The use of more than one antigen in a single assay allows the detection of individuals exposed to HTLV-I that have differing antibody profiles. For any given sera positive population, individuals will exhibit different immunogenic responses to viral antigens. Thus, an assay employing only one antigen may not detect all the exposed individuals. A single screening assay employing more than one antigen is needed to ensure all exposed individuals are detected.

SUMMARY OF THE INVENTION

The present invention provides a HTLV-I assay which meets the criteria referred to above. This invention encompasses a method for detecting exposure to HTLV-I comprising combining a body fluid containing an antibody with an amount of one or more recombinant polypeptide antigens selected from the group consisting of polypeptides encoded by all or part of the env, tax, and gag genes of HTLV-I effective to maximize the sensitivity and selectivity of an immunological assay, wherein the polypeptides are bound to a synthetic polymeric solid support; forming a detectable antibody-antigen binding pair between the antibody and polypeptide; and detecting the presence of the pair on the support.

A preferred solid support is a microtiter well.

Preferred polypeptide antigens include env B, $p40^{x}$, and p24 gag. The preferred polypeptide, env B, is a polypeptide that has a molecular weight of about 26,500 and has an amino acid sequence which includes a portion of the amino acid sequence, amino acids 158-308, encoded by the native HTLV-I env gene. The preferred polypeptide, $p40^{x}$, is a protein that has a molecular weight of about 40,000 and has an amino acid sequence which includes the amino acid sequence encoded by the native HTLV-I tax gene. The preferred polypeptide, p24 gag, is a protein that has a molecular weight of about 24,000 and has an amino acid sequence which includes a portion of the amino acid sequence encoded by the native HTLV-I gag gene.

A preferred embodiment of this invention uses at least three recombinant polypeptides in a single assay, where the three polypeptides are selected from the group consisting of polypeptides encoded by all or part of the env, tax, and gag genes of HTLV-I.

This invention also encompasses an assay kit for detecting HTLV-I antibodies comprising a solid support coated with an amount of at least three recombinant polypeptide antigens selected from the group consisting of polypeptides encoded by all or part of the env, tax, and gag genes of HTLV-I effective to maximize the sensitivity and selectivity of an immunological assay, wherein binding sites on the support that do not contain polypeptide antigens are blocked; antigen-antibody binding pair detection reagents; and developing reagents which provide a detectable signal from the detection reagents. A preferred assay kit includes the three polypeptides, env B, $p40^x$, and p24 gag, bound to a microtiter well. The kit may also include additional reagents which may provide various diluents or buffers needed to perform the assay. A preferred detection reagent is an enzyme-conjugated goat antihuman antibody.

Further, the present invention encompasses a competition immunoassay to detect the presence of an antibody to a HTLV-I antigen comprising preparing identical first and second dilutions of a body fluid containing an antibody; generating a detectable signal using the first dilution and a recombinant polypeptide antigen, wherein the antibody forms a detectable antigen-antibody binding pair and the antigen is selected from the group consisting of purified env B, $p40^x$, and p24 gag polypeptides; adding a known amount of a recombinant polypeptide antigen selected from the group consisting of purified env B, $p40^x$, and p24 gag polypeptides to the second dilution, wherein the antigen is the antigen used with the first dilution; generating a detectable signal using the second dilution, wherein the HTLV-I antibody forms a detectable antigen-antibody binding pair with the antigen used with the first dilution; and determining the presence of the HTLV-I antibody in the first sample by comparing the difference of the signals of the first and second dilutions.

The present invention additionally encompasses an immunological composition for use in assays to detect a HTLV-I antibody comprising a solid support; an amount of at least three purified recombinant polypeptide antigens selected from the group consisting of polypeptides encoded by all or part of the env, tax, and gag genes of HTLV-I effective to maximize the sensitivity and selectivity of an immunological assay; and an amount of a blocking agent bound to said support that is sufficient to minimize nonspecific binding.

Preferably, both the screening and competition assays incorporate recombinant polypeptides produced by transformed E. coli. The preferred polypeptides are p24 gag, env B, and $p40^{x}$. These polypeptides are preferably purified in order to provide the optimized sensitivity and selectivity for the assay. As used herein, the term "purified" refers to polypeptides that are free from contaminating materials which would interfere in an immunoassay. For example, contaminating E. coli proteins might result in a false positive result if a particular sample contained antibodies to E. coli proteins.

The preferred amounts of each antigenic polypeptide for use in an immunoassay or assay kit either individually or as a combination of two or more antigenic polypeptides bound to the same support or to the same microtiter well, are about 1.89×10^{-13} to 3.78×10^{-11} moles env B, 1.25×10^{-13} to 2.50×10^{-11} moles $p40^{x}$, or 2.09×10^{-13} to 8.34×10^{-11} moles p24 gag. Particularly preferred amounts of antigenic polypeptides for the above assays are about 3.78×10^{-13} to 9.45×10^{-12} moles env B, 2.5×10^{-13} to 6.25×10^{-12} moles $p40^{x}$, or 4.17×10^{-13} to 2.08×10^{-11} moles p24 gag. The most preferred amounts of antigenic polypeptides are 1.89×10^{-12} moles env B, 1.25×10^{-12} moles $p40^{x}$, or 1.04×10^{-11} moles p24 gag.

The preferred amounts of antigenic peptide may be added to a microtiter well as a solution. Preferably, about 200 microliters of a solution having a concentration of about 0.95 to 189 nmolar env B, 0.625 to 125 nmolar $p40^x$, or 1.05 to 417 nmolar p24 gag may be added to a microtiter well. Particular preferred concentrations for a 200 microliter solution are 1.89 to 47.3 nmolar env B, 1.25 to 31.3 nmolar $p40^x$, or 2.08 to 104.3 nmolar p24 gag. The most preferred concentrations for a 200 microliter solution are 9.5 nmolar env B, 6.25 nmolar $p40^x$ or 52.0 nmolar p24 gag.

Other aspects and advantages of the present invention will be evident after consideration of the following description of the invention and the illustrative examples of the practice of this invention.

DESCRIPTION OF THE DRAWINGS

Figures 1A-1C illustrates a composite synthetic/ native DNA sequence encoding the amino acid sequence of the antigenic polypeptide env B, Figures 2A-2B illustrates a synthetic DNA sequence encoding the amino acid sequence of the antigenic polypeptide p24 gag and Figures 3A-3C illustrates a composite synthetic/native DNA sequence encoding the amino acid sequence of the antigenic polypeptide $p40^x$.

DESCRIPTION OF THE INVENTION

The HTLV-I genome encodes at least three proteins that may be antigenic in humans, gag, env, and tax proteins. Preferably, a test for exposure to HTLV-I includes antigenic proteins encoded by these genes in order to provide a very sensitive and specific test.

Table 1 illustrates various immunogenic responses identified from 46 positive serum samples. The 46 samples were initially identified as positive using a multiple antigen assay containing three

antigenic polypeptides, p24 gag, env B, and $p40^X$. Each of the samples were then assayed using a single antigenic polypeptide. The results indicate different individuals generate varying immunogenic responses to HTLV-I exposure. An immunoassay employing only a single antigen would not be able to accurately identify all infected serum samples.

TABLE 1

POSITIVE SAMPLES FROM A SERUM PANEL

Antigen Polypeptide(s)	Samples Having a Positive Antibody Response
env B + p24 gag; no $p40^X$	17
env B + p24 gag + $p40^X$	15
env B only; no p24 gag or $p40^X$	12
env B + $p40^X$; no p24 gag	1
p24 gag only	1

Recombinant HTLV-I polypeptides suitable for use in this invention may be prepared by employing known recombinant DNA technologies. Briefly, synthetic DNA sequences to encode all or part of the antigenic polypeptides were designed and prepared to optimize expression of the encoded polypeptides in E. coli. The actual DNA sequences employed were derived from the native HTLV-I amino acid sequences rather than from the native nucleic acid sequences. This derivation technique allowed the DNA sequence to be designed for optimal expression in E. coli. The DNA sequences used to express the desired polypeptides may include fragments of native DNA that were isolated from natural

sources as well as fragments of synthetic DNA that were laboratory-made. The complete sequences were assembled from the different fragments using well established techniques.

The complete DNA sequences were incorporated into expression vectors and the vectors were transformed into E. coli cells. The transformed cells were then used to express the desired polypeptides encoded by the complete DNA sequences. Upon isolation using known methods, the identities of the expressed recombinant polypeptides were confirmed both biochemically and immunologically.

Alternatively, HTLV-I recombinant antigens suitable for use in this invention may be obtained from Triton Biosciences, Alameda, California. The commercially available env polypeptide, p21e/gp46, is a recombinant fusion protein with a molecular weight of 38,000 which includes a majority of the p21e amino acid sequence and a substantial part of the gp46 amino acid sequence. The commercially available polypeptide, fusion p24 gag, is a fusion protein with a molecular weight of 40,000 which includes the entire p24 gag amino acid sequence. The commercially available polypeptide, fusion $p40^{x}$, is a fusion protein with a molecular weight of 42,000.

An assay according to this invention may use about 5 to 2000 ng of purified antigen bound to a suitable solid support. Preferred amounts of each antigen are 10 to 250 ng of env B, 10 to 250 ng of $p40^{x}$ and 10 to 500 ng of p24 gag, the most preferred amounts are 50 ng of env B, 50 ng of $p40^{x}$ and 250 ng of p24 gag. Amounts of bound antigen less than 10 ng appear to be about the lower limit of sensitivity for detection of HTLV-I antibodies in routine samples. Amounts of bound antigen exceeding 250 ng appear to be about the upper limit of selectivity because the binding specificity of

HTLV-I antibodies, compared to other antibodies in the sample, begins to significantly decrease.

To adsorb these amounts of antigen to the solid support, specific concentrations that are used to coat the support as a 200 microliter solution are preferably 0.95 to 189 nmolar env B, 0.625 to 125 nmolar $p40^{x}$, and 1.05 nmolar to 417 nmolar p24 gag, particularly preferred concentrations are 1.89 to 47.3 nmolar env B, 1.25 to 31.3 nmolar $p40^{x}$, and 2.08 to 104.3 nmolar p24 gag, the most preferred concentrations are 9.5 nmolar env B, 6.25 nmolar $p40^{x}$, and 52.0 nmolar p24 gag.

The following examples are for illustrative purposes only and are not intended to limit the scope of the claims. These examples illustrate screening and competition assay protocols based on the specific immunoreactivity of HTLV-I antibodies with recombinant antigens bound to a solid support. Examples 1 to 4 describe the preparation of recombinant polypeptide antigen coated microtiter wells and procedures to test sera samples for HTLV-I antibodies using these coated wells. Example 5 describes competition assays which preferably provide confirmatory results for the presence of a particular HTLV-I antibody in a given sample. Example 6 describes immunoblot assay protocols in which the antigenic polypeptides are bound to a nitrocellulose solid support. Examples 7 to 9 describe procedures to prepare p24 gag, env B, and $p40^{x}$ recombinant antigenic polypeptides, respectively. Examples 10 to 12 describe procedures to purify the HTLV-I antigenic polypeptides used in this invention.

Example 1

Presenting env Polypeptides to a Well

Two antigenic env polypeptides were used to prepare microtiter wells for detecting HTLV-I antibodies. The HTLV-I 38 kilodalton polypeptide, p21e/gp46, was obtained commercially from Triton Biosciences, Alameda, California. The env B polypeptide described in Example 8 was expressed in E. coli and purified as described infra. Either env polypeptide was placed in 8 M Guanidine-HCl and 50 mM Glycine, pH 3.0, at a concentration of 2 mg per ml. This concentrate was then diluted to 0.25 ng/µl in 4 M Guanidine-HCl and 50 mM Tris-HCl, pH 7.4, to make an immunoassay microtiter well adsorption solution and 200 µl of the adsorption solution was added to each microtiter well of an Immulon IV Removawell strip (Dynatech Laboratories, Inc., Chantilly, Virginia, Catalog Number 011-010-6404). The microtiter wells containing the adsorption solution were covered and incubated for 12 hours at 37°C in a humidified incubator. After the incubation period, the solution in the microtiter wells was decanted and the microtiter wells were inverted and patted on an absorbent pad to remove excess solution remaining in the microtiter wells. A blocking solution (250 µl, 2% alkaline casein, 2% glycerol, 10% sucrose in TEN buffer: 150 mM NaCl, 10 mM tetrasodium EDTA, 1:10000 (w/v) thimerosal, and 50 mM Tris-HCl, pH 7.2 to 7.6, as described in Clinica Chemica ACT 193:123 (1982)) was added to each well. The microtiter wells were covered and incubated with the blocking solution for 12 hours at 37°C in a humidified incubator. After blocking, the coated and blocked microtiter wells were decanted and patted as above. The microtiter wells were then inverted and dried for 12 hours at 4°C. Once the coated and blocked microtiter wells had been dried, they were stored at -20°C until used.

Antibodies to the HTLV-I env polypeptide in human serum or plasma were detected with an enzyme linked detection antibody by adding 4 µl of the serum or plasma to 2 ml of sample diluent (25% normal goat serum, 0.1% polyoxyethylenesorbitan monolaurate (Tween-20), in TEN buffer) and 150 µl of the diluted serum or plasma was added to each microtiter well at room temperature. The microtiter wells were covered and incubated for 2 hours at 37°C in a humidified incubator. The diluted serum or plasma was decanted from the microtiter well and the microtiter wells were patted on an absorbent pad to remove any remaining solution.

The wells were washed three successive times with wash solution (0.01% Tween-20 in TEN buffer). Each wash step involved squirting the wash solution from a 500 ml squirt bottle into the microtiter well until full, decanting the wash solution and patting the well on an absorbent pad to remove the excess wash solution.

Horseradish peroxidase-conjugated goat antihuman antibody (BioRad Laboratories, Richmond, California) was diluted 1:10000 into conjugate diluent (0.1% alkaline-treated casein in TEN buffer) and 150 µl of the diluted conjugate was added to each microtiter well. The microtiter wells were then covered and incubated for 30 minutes at 37°C. After incubation with the conjugated antibody, the microtiter wells were washed three times as above. A 10 mg o-phenylenediamine tablet (OPD2, Chemicon, El Segundo, California) was dissolved in 10 ml OPD buffer (OPD4, Chemicon), warmed to room temperature to make a substrate solution and 150 µl of the substrate solution was added to each well. Each well was covered and incubated for 30 minutes at 4°C. After incubation for 30 minutes, color development in the microtiter wells was stopped by adding 2.5 M sulfuric acid (75 µl) to each well. The amounts of color development in the microtiter wells were

quantitated by reading the optical density at 490 nm minus the optical density at 650 nm.

Using the microtiter wells and assay procedures described above, thirty-one HTLV-I positive serum or plasma samples were examined. Of these samples, twenty were positive for antibodies to the HTLV-I env protein by radioimmunoprecipitation analysis (RIPA). The RIPA analysis was performed following procedures described by Slamon, et al., Science, 226:61-65 (1984).

Seventeen of the RIPA positive samples reacted positively in the microtiter well assay for env B. Twenty-one of the HTLV-I positive samples reacted in a microtiter assay for p21e/gp46. Three of those samples that reacted weakly positive were negative by RIPA. The remaining samples scored with values similar to env B and were in correspondence with env B for reactivity by RIPA.

Table 2 illustrates the preferred range of the amount of antigenic polypeptide env B to incorporate in the assay to provide the greatest sensitivity and selectivity.

TABLE 2

Experiment Number	6	8	12	14	20	27
Amount of Antigen	10ng	10ng	50ng	50ng	250ng	250ng
Antigen Buffer	4 M Guan-TRIS	4 M Guan-TRIS	4 M Guan-TRIS	4 M Guan-TRIS	4 M Guan-TRIS	4 M Guan-TRIS
Antigen Volume	150µl	200µl	100µl	150µl	100µl	200µl
Antigen Temperature	37°C	22°C	4°C	37°C	4°C	22°C
Antigen Time	24 hrs	12 hrs	12 hrs	6 hrs	24 hrs	6 hrs
Block Solution	ATC/X-100	ASGT	ATC/X-100	ASGT	ASGT	ATC/X-100
Block Volume	200µl	200µl	300µl	300µl	250µl	250µl
Block Time	12 hrs	12 hrs	24 hrs	24 hrs	36 hrs	36 hrs
Block Temperature	22°C	37°C	37°C	4°C	22°C	4°C
Dry Time	24 hrs	36 hrs	12 hrs	24 hrs	12 hrs	36 hrs
Dry Temperature	22°C	37°C	22°C	37°C	37°C	22°C
*P/N env B	5.6	6.2	12.6	10.0	8.1	9.6

*P/N is the value of the average of five optical density measurements for HTLV-I positive serum divided by the average of five optical density measurements for HTLV-I negative serum. The protocols used to present varying amounts of antigen to the wells were not identical for each concentration.

Example 2

Presenting p24 gag Polypeptide to a Well

The p24 gag polypeptide described in Example 7 was expressed in E. coli and purified as described infra. The polypeptide was placed in 1 mM dithiotheitol (DTT) and 50 mM Tris-HCl, pH 7.5, at a concentration of 2 mg per ml. This concentrate was then diluted to 1.25 ng/µl in 100 mM NaCl and 50 mM Tris-HCl, pH 7.4, to make a immunoassay microtiter well adsorption solution and 200 µl of the adsorption solution was added to each microtiter well of an Immulon IV Removawell strip (Dynatech Laboratories, Inc., Chantilly, Virginia, Catalog Number 011-010-6406.) The microtiter wells containing the p24 gag solution were covered and incubated for 12 hours at 22 °C in a humidified incubator. After the incubation period, the solution in the microtiter wells was decanted and the microtiter wells were inverted and patted on an adsorbent pad to remove excess p24 gag solution remaining in the microtiter wells. A blocking solution (200 µl, 2% alkaline-treated casein, 2% glycerol, 10% sucrose in TEN buffer: 150 mM NaCl, 10 mM tetrasodium EDTA, 1:10000 (w/v) thimerosal, and 50 mM Tris-HCl, pH 7.2 to 7.6) was added to each well. The microtiter wells were covered and incubated with the blocking solution for 12 hours at 37°C in a humidified incubator. After blocking, the p24 gag coated and blocked microtiter wells were then inverted and dried for 12 hours at 4°C. Once the p24 gag coated and blocked microtiter wells had been dried, they were stored at -20°C until used.

Antibodies to p24 gag in human serum or plasma were detected using the same assay protocol as that for detection of antibodies to env polypeptides described in Example 1.

Table 3 illustrates t preferred range of the amount of antigenic polypeptid 24 gag to incorporate in the assay to provide the greatest sensitivity and selectivity.

TABLE 3

Experiment Number	2	4	10	19	23	25
Amount of Antigen	10ng	10ng	50ng	50ng	250ng	250ng
Antigen Buffer	TRIS-NaCl	TRIS-NaCl	TRIS-NaCl	TRIS-NaCl	TRIS-NaCl	TRIS-Na
Antigen Volume	150µl	200µl	150µl	100µl	200µl	100µl
Antigen Temperature	22°C	4°C	22°C	37°C	4°C	37°C
Antigen Time	12 hrs	6 hrs	24 hrs	6 hrs	24 hrs	12 hrs
Block Solution	ATC/X-100	ASGT	ASGT	ATC/X-100	ATC/X-100	ASGT
Block Volume	250µl	250µl	200µl	200µl	300µl	300µl
Block Time	24 hrs	24 hrs	36 hrs	36 hrs	12 hrs	12 hrs
Block Temperature	22°C	37°C	4°C	37°C	4°C	22°C
Dry Time	12 hrs	24 hrs	12 hrs	36 hrs	24 hrs	36 hrs
Dry Temperature	4°C	22°C	22°C	4°C	4°C	22°C
P/N p24 gag	4.53	5.16	8.03	4.78	7.02	11.67

*P/N is the value of the average of five optical density measurements for HTLV-I positive serum divided by the average of five optical density measurements for HTLV-I negative serum. The protocols used to present varying amounts of antigen to the wells were not identical for each concentration.

Table 4 also illustrates the preferred range of the amount of antigenic polypeptide used to provide the optimal sensitivity and selectivity in an assay. The data clearly illustrates lesser amounts, 10 ng or less, suffer from lack of sensitivity where as higher amount show decreasing selectivity. The optical density measurements were made by reading the 96 well microtiter plate on a Vmax Kinetic Microplate Reader, (Molecular Devices Corp., Menlo Park, CA). The raw optical density determination for each well was made by calculating the difference in optical density from a measurement made at lambda = 490 versus a measurement made at lambda = 650. The different optical density values were then normalized to a background level by subtracting the optical density measurement for a microtiter well containing only developed and stopped substrate reagent (the substrate reagent was stopped with 2.5 M H_2SO_4) no added serum or secondary conjugate.

TABLE 4

Optical Density (450-650) at Different Concentrations of p24 gag

(Amount of 24 gag in well)		8ng	16ng	31ng	62ng	125ng	250ng	500ng	1000ng
Serum Dilution	None	0.383,0.236	0.736,0.453	1.004,0.655	1.082,1.011	1.145,1.532	1.007,1.734	1.028,1.574	0.907,1.649
(No dilution= 1/500 sample/ dilution volume)	1:2	0.202,1.113	0.431,0.259	0.547,0.390	0.637,0.571	0.688,0.971	0.582,0.980	0.592,0.953	0.560,0.955
	1:4	0.110,0.060	0.231,0.137	0.206,0.212	0.358,0.312	0.351,0.513	0.307,0.581	0.300,0.513	0.290,0.511
	1:8	0.62, 0.033	0.115,0.065	0.147,0.107	0.183,0.161	0.182,0.277	0.147,0.293	0.149,0.271	0.149,0.264
	1:16	0.028,0.014	0.059,0.028	0.076,0.051	0.101,0.087	0.092,0.131	0.076,0.153	0.074,0.149	0.076,0.134
	1:32	0.013,0.007	0.031,0.013	0.037,0.027	0.047,0.039	0.046,0.061	0.037,0.073	0.037,0.074	0.038,0.071

data from serum sample numbers 9050 and 0875.

Example 3

Presenting $p40^X$ to a Well

The $p40^X$ polypeptide described in Example 9 was expressed in E. coli and purified as described infra. The polypeptide was placed in 8 M Guanidine-HCl, 100 mM dithiotheitol (DTT) and 50 mM Tris-HCl, pH 7.5, at a concentration of 2 mg per ml. This concentrate was then diluted to 0.34 ng/µl in 4 M Guanidine-HCl and 50 mM Tris-HCl, pH 7.4, to make an immunoassay microtiter well adsorption solution and 200 µl of the solution was added to each microtiter well of an Immulon IV Removawell strip (Dynatech Laboratories, Inc., Chantilly, Virginia, Catalog Number 011-010-6404). The microtiter wells containing the $p40^X$ solution were covered and incubated for 12 hours at 4°C in a humidified incubator. After the incubation period, the solution in the microtiter wells was decanted and the microtiter wells were inverted and patted on an absorbent pad to remove excess solution remaining in the microtiter wells. A blocking solution, (200 µl, 2% alkaline-treated casein, 2% glycerol, 10% sucrose in TEN buffer: 150 mM NaCl, 10 mM tetrasodium EDTA, 1:10000 (w/v) thimerosal, and 50 mM Tris-HCl, pH 7.2 to 7.6) was added to each well. The microtiter wells were covered and incubated with the blocking solution for 12 hours at 4°C in a humidified incubator. After blocking, the coated and blocked microtiter wells were decanted and patted as above. The microtiter wells were then inverted and dried for 36 hours at 4°C. Once the coated and blocked microtiter wells had been dried, they were stored at -20°C until used.

Antibodies to $p40^X$ in human serum or plasma were detected using the same protocol as that for detection of antibodies to env peptides described in Example 1.

Table 5 illustrates the preferred range of the concentration of antigenic polypeptide $p40^X$ to incorporate in the assay to provide the greatest sensitivity and selectively.

TABLE 5

Experiment Number	6	8	12	14	20	27
Amount of Antigen	10ng	10ng	50ng	50ng	250ng	250ng
Antigen Buffer	4 M Guan-TRIS	4 M Guan-TRIS	4 M Guan-TRIS	4 M Guan-TRIS	4 M Guan-TRIS	4 M Guan-TRIS
Antigen Volume	150µl	200µl	100µl	150µl	100µl	200µl
Antigen Temperature	37°C	22°C	4°C	37°C	4°C	22°C
Antigen Time	24 hrs	12 hrs	12 hrs	6 hrs	24 hrs	6 hrs
Block Solution	ATC/X-100	ASGT	ATC/X-100	ASGT	ASGT	ATC/X-100
Block Volume	200µl	200µl	300µl	300µl	250µl	250µl
Block Time	12 hrs	12 hrs	24 hrs	24 hrs	36 hrs	36 hrs
Block Temperature	22°C	37°C	37°C	4°C	22°C	4°C
Dry Time	24 hrs	36 hrs	12 hrs	24 hrs	12 hrs	36 hrs
Dry Temperature	22°C	37°C	22°C	37°C	37°C	22°C
P/N $p40^x$	2.1	2.6	6.9	5.8	6.7	5.5

*P/N is the value of the average of five optical density measurements for HTLV-I positive serum divided by the average of five optical density measurements for HTLV-I negative serum. The protocols used to present varying amounts of antigen to the wells were not identical for each concentration.

Example 4

Multiple Antigen Assay

The p24 gag polypeptide, env B polypeptide, p21e/gp46, and the p40x polypeptide prepared as described in Examples 7, 8 and 9 and purified as described infra, were placed in 4 M guanidine-HCl to make a single adsorption solution with polypeptide concentrations of 250 ng/200 µl p24 gag, 50ng/200 µl p21e/gp46, and 50 ng/200 µl $p40^{X}$, respectively, and 200 µl of the adsorption solution was added to each microtiter well of an Immulon IV Removawell strip (Dynatech Laboratories, Inc. Chantilly, Virginia, Catalog Number 011-010-6404). The microtiter wells containing the adsorption solution were incubated for 12 hours at 37°C. After the adsorption, the adsorption solution in the microtiter wells was decanted and the microtiter wells were inverted and patted on an absorbent pad to remove any excess adsorption solution remaining in the microtiter wells. A blocking solution (250 µl, 2% alkaline-treated casein, 2% glycerol, 10% sucrose in TEN buffer: 150 mM NaCl, 10 mM tetrasodium EDTA, 1:10000 (w/v) thimerosal, and 50 mM Tris HCl, pH 7.2 to 7.6) was added to each well. The microtiter wells were covered and incubated with the blocking solution for 12 hours at 37°C in a humidified incubator. After blocking, the antigen coated wells were decanted and patted as above. The microtiter wells were then inverted and dried for 12 hours at 4°C. Once the antigen coated and blocked wells had been dried, they were stored at -20°C until used.

Antibodies to the HTLV-I antigens in human serum or plasma were detected using the same protocol as that for detection of env antibodies in human serum or plasma. Fifty-two HTLV-I positive sera containing antibodies to p24 gag, env B, or $p40^{X}$ were examined for

reactivity in the multiple antigen assay. All fifty-two samples were identified by the multiple antigen assay.

Table 6 illustrates the optical density measurements for a multiple antigen assay using the three antigenic polypeptides, p24 gag, env B, and $p40^{x}$. Optical density measurements for assays using each antigenic polypeptide individually are also given in Table 6.

TABLE 6

Serum Diluent	1%NGS/0.1%T-20	1%NGS/0.1%T-20	1%NGS/0.1%T-20	1%NGS/0.1%T-20	1%NGS/0.1%T-20	1%NGS/0.1%
Serum Dilution	1/5	1/50	1/500	1/5	1/50	1/500
Serum Volume	150µl	150µl	150µl	150µl	150µl	150µl
Serum Temperature	37°C	4°C	22°C	4°C	22°C	37°C
Serum Time	1 hr	2 hrs	3 hrs	3 hrs	1 hr	2 hrs
Serum and 2° Washes	3 x 5'	3 quick	3 x 3'	3 x 3'	3 x 5'	3 quick
2° Volume	200µl	100µl	150µl	200µl	100µl	150µl
2° INC. Time	30'	60'	120'	60'	120'	30'
Substrate Volume	200µl	150µl	100µl	100µl	200µl	150µl
Substrate Temperature	37°C	22°C	4°C	37°C	22°C	4°C

Substrate Time	30'	20'	10'	20'	10'	30'
P/N Multi	1.4	3.1	17.8	1.5	8.1	41.6
P/N p24 gag	1.4	3.6	25.9	1.6	7.1	46.0
P/N env B	1.3	2.4	13.1	1.5	5.7	35.0
P/N $p40^{x}$	1.1	1.5	5.8	1.1	2.8	19.7

*P/N is the value of the average of five optical density measurements for HTLV-I positive serum divided by the average of five optical density measurements for HTLV-I negative serum. The protocols used to present varying amounts of antigen to the wells were not identical for each concentration.

Other commercially available tests using viral lysate antigen (DuPont and Abbott) did not identify either 2 samples (DuPont) or 1 sample (Abbott). This suggested that the commercially available tests are not as sensitive as the recombinant multiple antigen assay.

Example 5

Competition Assays

Commercial Western blots were purchased either from DuPont or Epitope. The Western blots were performed as described in the package insert except that in selected samples 2 µg of recombinant p24 gag was added to the serum diluent at the same time the serum was added to the serum diluent. After developing the Western blot following the manufacturer's protocol, those samples which were previously known to be positive for p24 gag reacted visibly with the Western blot strip when no recombinant p24 gag was added. However, if the recombinant p24 gag was added at the time of addition of the serum to the serum diluent no or very little signal was visibly seen. This suggests that recombinant p24 gag may be used in a competition Western blot format and that recombinant p24 gag is immunologically equivalent to viral derived p24 gag for serum antibodies.

Using an assay protocol as described in Example 1 for detection of serum antibodies to env polypeptides 2 µg of recombinant p24 gag was added to the HTLV-I positive serum immediately prior to addition of the serum to the sample diluent. The signal for HTLV-I positive serum without the addition of recombinant p24 gag was 1.860 O.D. Addition of recombinant p24 gag reduced the signal to 0.294 O.D. The O.D. signals for normal human serum were 0.123 without the addition of p24 gag and 0.114 O.D. with the addition of recombinant p24 gag.

Using an assay protocol described in Example 1 to detect serum antibodies to env polypeptides, preincubation of the HTLV-I positive serum with 2 µg of purified recombinant env B and then assaying the sample reduces the signal from 2.672 O.D. to 0.045 O.D. Addition of E. coli extract or purified alpha consensus interferon did not reduce the signal. This indicated that the observed competition was specific for antibodies reactive with the HTLV-I component of env B and not to the fusion leader sequence component or potentially contaminating E. coli proteins. No change in the ELISA signal was observed when normal human serum was used. The observed signal was 0.016 O.D. with env B and 0.016 O.D. without env B.

A RIPA analysis was performed as described by Slamon, et al., Science, 226: 61-65 (1984). Either recombinant p24 gag or $p40^{x}$ was added to the radiolabelled lysate at the same time as the HTLV-I positive serum was added. The RIPA procedure was followed as described by Slamon, et al. Exposure of the autoradiographs of serum co-incubated with the recombinant HTLV-I antigenic polypeptides resulted in a significant visible decrease in the intensity of the associated antigen band on the gel. This indicated that the recombinant polypeptides added during the serum incubation competed with the radiolabelled HTLV-I antigen in the lysate for serum HTLV-I antibodies. This also indicated that the recombinant polypeptides are immunologically equivalent to the viral-derived antigens using HTLV-I positive serum.

Example 6

Immunoblot Assays

The antigenic polypeptides are placed in a suitable buffer such as 8 M Guanidine-HCl in distilled

water, 4 M Guanidine-HCl in distilled water, or distilled water. The concentrations are selected to optimize the assay selectivity and sensitivity, typical concentrations are about 20 ng/µl to 500 ng/µl.

Commercially available nitrocellulose solid supports which may be used include: 1) nitrocellulose 0.45 µm, 0.2 µm, 0.1 µm, (Schleicher and Schuell); 2) nitrocellulose 0.45 µm (Micron Separation Inc.); 3) Nytran 0.45 µm and 0.65 µm (Schleicher and Schuell); 4) Biotran S 0.2 µm (ICN); 5) plastic-backed nitrocellulose 0.45 µm (Schleicher and Schuell); 6) ZetaProbe (BioRad Laboratories); 7) Immobilon P 0.45 µm (Millipore, Bedford, MA); 8) Immunodyne 0.45 µm (Pall Biosupports); and 9) GeneScreen or GeneScreen Plus (New England Nuclear). Generally each antigenic polypeptide concentrate is contacted with the solid support and allowed to dry.

The coated supports are further treated with a blocking solution (3% Carnation nonfat dry milk, 0.3% Tween-20, in Dulbecco's phosphate-buffered saline, PBS, Gibco Laboratories, Grand Island, NY) by placing the supports and blocking solution in a multiple well tray. The tray is typically shaken at 60 RPM on a rotary shaker (Junior Orbit Shaker, Labline) at room temperature for one hour. The blocking solution is then aspirated from the tray wells. If desired, the strips may be removed and placed antigen side up on blotting paper, such as 3MM, Whatman, for approximately 30 minutes until dry. The strips may then be stored sandwiched between blotting paper at 4°C in the dark.

To run a typical immunoblot assay, a sample is diluted in the same solution as the blocking solution and then added to each tray well containing a coated and blocked support. The sample and support are shaken on a rotary shaker generally at room temperature for about one to five hours. The excess sample containing the

serum or plasma is then aspirated. A wash solution, such as 0.3% Tween-20 in PBS is added to each well, the wells are rocked back and forth several times and the wash solution is then aspirated. This wash and aspiration procedure is repeated, if desired. The supports are then washed again and excess wash solution is aspirated.

To generate a visible signal the support is treated with a solution of purified goat antihuman horseradish peroxidase conjugated antibody (BioRad, 1:2000 dilution of conjugate into sample diluent). The support is shaken on a rotary shaker generally for about one hour at room temperature. The supports are then aspirated and washed, if desired. The supports are washed an additional time with water and aspirated.

A substrate developing solution such as 78.6 ml of deionized water added to 1.334 ml of 3 M ammonium acetate, pH 5.5, 80 µl of 30% hydrogen peroxide, 2 ml of 10 mg/ml 3,3'-diaminobenzidine tetrahydrochloride (available from Sigma, St. Louis, MO) is then added to the support. The support is incubated for about 5 to 30 minutes at room temperature until adequate color develops. The substrate developing reaction is stopped by rinsing the strip in the well with deionized water.

Six HTLV-I positive sera were tested using antigenic polypeptides bound to nitrocellulose. All six HTLV-I positive sera reacted visibly with purified p24 gag at all concentrations while HTLV-I negative sera did not react significantly at any concentration. Two HTLV-I positive sera reacted best with respect to negative serum at the 10 ng concentration for env B. Other concentrations of the purified env B gave a visible signal for the negative serum. Three HTLV-I positive sera reacted significantly for $p40^{x}$ at concentrations of 10 and 50 ng while the negative sera reacted only weakly.

In all cases, when the antigen is initially taken up in 4 M or 8 M Guanidine-HCl the signal was improved over presentation of the antigen to the solid support in distilled water.

Example 7

Preparation of p24 gag

The DNA sequence encoding the antigenic polypeptide was constructed from three laboratory produced oligonucleotides.

The following oligonucleotide, (DNA sequences are shown as a single strand for simplicity)

```
          10         20         30         40         50
 AGCTTGATG GAAAGCTTGC ACCATCAACA GCTGGACAGC CTGATTTCCG
 H          HA
 I          IL
 N          NU
 3          31

          60         70         80         90        100
AAGCGGAAAC ACGCGGTATC ACCGGCTACA ACCCGCTGGC GGGTCCACTG

         110        120        130        140        150
CGTGTTCAAG CCAACAATCC TCAGCAACAG GGTCTGCGTC GCGAATATCA

         160        170        180        190        200
ACAGCTGTGG CTCGCGGCAT TCGCTGCGCT GCCGGGCTCT GCGAAAGATC
```

```
      210         220         230         240
CGAGCTGGGC TTCTATTCTG CAAGGTCTCG AGTAATAACC AGGATC
                           XT                  BB
                           HA                  AI
                           OQ                  MN
                           11                  11
```

was prepared utilizing known procedures described in U.S. Patent 4,652,639. This patent is incorporated by reference herein for the purpose of providing information relating to the method employed for manufacturing DNA sequences.

The nucleotide fragments were synthesized on an Applied BioSystems 380B DNA synthesizer following operating parameters established by the manufacturer. In general, the oligonucleotide fragments were synthesized by sequentially condensing protected nucleic acids using a three-step procedure and several intermediate washes. Polymer bound dimethoxytrityl protected nucleoside in a sintered glass funnel was first stripped of its 5'-protecting group (dimethoxytrityl) using 2% trichloroacetic acid in methylene chloride for 1.5 minutes. The polymer was then washed with methanol, tetrahydrofuran and acetonitrile. The washed polymer was rinsed with dry acetonitrile, placed under argon and then treated in the condensation step as follows: a solution of 10 mg tetrazole in 0.5 ml of acetonitile was added to the reaction vessel containing polymer; 30 mg of a protected nucleoside phosphoramidite in 0.5 ml of acetonitrile was added; the mixture was agitated and allowed to react for 2 minutes; the reactants were removed by suction; and the polymer was rinsed with acetonitrile. This was followed by an oxidation step wherein a solution containing 0.1 molar iodine in one ml of 2,6-lutidine/water/tetrahydrofuran (1:2:2) was reacted with

the polymer bound oligonucleotide chain for 2 minutes, followed by an acetonitrile rinse. The cycle was repeated until the desired oligonucleotide sequence was obtained.

After condensing, the final nucleotide chain was treated with thiophenol/dioxane/triethylamine (1:2:2) for 45 minutes at room temperature. Then, after rinsing with dioxane, methanol and diethyl ether, the oligonucleotide was cleaved from the polymer using concentrated ammonia and the oligonucleotide solution was extracted four times with 1-butanol. The solution was loaded into a 20% polyacrylamide 7 M urea electrophoresis gel and, after running, the appropriate oligomer-containing band was isolated.

Appropriate oligomers were treated with polynucleotide kinase to introduce 5' phosphate moieties as described in Maniatis, et al., Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory (1982). Oligomer duplexes were formed by mixing equal molar quantities of the appropriate single-strand oligomers in water, placing the mixture in a boiling water bath for 5 minutes, and then slowly cooling to 4°C. The hybridized oligomer duplexes were then covalently connected with bacteriophage T4 ligase to form the correct duplex structures for molecular cloning. The duplex synthetic DNA constructs were isolated by 8-10% PAGE and passive elution.

After isolation, the synthetic DNA sequence was then inserted as a HindIII/BamHI fragment into plasmid pCFM1156, described below, to give plasmid pADK1001. The plasmid pCFM1156 was digested with restriction enzymes HindIII and BamHI, and isolated by agarose gel electrophoresis using a NA-45 membrane (as described in Schleicher & Schuell Applications Update No. 364). The purified vector was mixed with an equal molar quantity of the above synthetic DNA segment,

ligated using T4 ligase, and transformed into E. coli host cell FM6, described below. Transformants were plated onto Luria Broth/kanamycin agar plates and grown for approximately 24 hours at 30°C. Candidate colonies were then picked and grown in 10 ml of Luria Broth/ kanamycin (50-µg/ml) overnight at 30°C. Candidates were diluted 1:50 into fresh Luria Broth/kanamycin and grown until the optical density at 600 nm was 0.2. At that time, the cultures were heated to 42°C and grown for an additional 5 hours. Samples of the induced whole cells were run on SDS-PAGE to look for protein expression. Cells expressing the desired protein had a band at the correct molecular weight which was not present in cells not containing the desired construct.

The plasmid pCFM1156 is prepared from the known plasmid pCFM836. The preparation of plasmid pCFM836 is described in U.S. Patent 4,710,473, the relevant portions of the specification, particularly examples 1 to 7, are hereby incorporated by reference. To prepare pCFM1156 from pCFM836, the two endogonous NdeI sites are cut, the exposed ends are filled with T4 polymerase and the filled ends are blunt-end ligated.

The resulting plasmid is then digested with ClaI and KpnI and the excised DNA fragment is replaced with a DNA oligionucleotide of the following sequence:

```
          10         20         30         40         50
     ClaI
5'CGATTTGA TTCTAGAAGG AGGAATAACA TATGGTTAAC GCGTTGGAAT
3'  TAAACT AAGATCTTCC TCCTTATTGT ATACCAATTG CGCAACCTTA

     KpnI
TCGGTAC3'
AGC    5'
```

The construction of the DNA sequence coding for the polypeptide p24 gag required preparing, as described above, a second oligonucleotide having the following sequence.

```
          10         20         30         40         50
 AGCTTAACT CGAGGAACCG TACCACGCCT TCGTGGAACG TCTGAACATC
HA       XT
IL       HA
NU       OQ
31       11

          60         70         80         90         100
GCTCTGGATA ATGGCCTGCC GGAAGGTACC CCGAAAGATC CTATTCTGCG

         110        120        130        140        150
CAGCCTGGCG TACAGCAACG CGAACAAAGA ATGTCAAAAA CTGCTGCAAG

         160        170        180        190        200
CTCGTGGTCA CACAAATAGC CCGCTGGGCG ATATGCTGCG TGCATGCCAA

         210        220        230        240
ACCTGGACTC CGAAAGATAA GACCAAAGTG CTGTAAGGAT C
                                            BB
                                            AI
                                            MN
                                            11
```

This second oligonucleotide was inserted into plasmid pADK1001 by digesting the oligonucleotide with XhoI and BamHI and ligating the XhoI/BamHI fragment into plasmid pADK1001 which had also been digested with XhoI and BamHI to give the plasmid pADK1007. The conditions to transform the host strain, FM6, with pADK1007 were similar to the conditions used with pADK1001, described above.

The construction of the DNA sequence coding for the polypeptide p24 gag was completed by inserting a third oligonucleotide into plasmid pADK1007. The third oligonucleotide of the following sequence was prepared as described above.

```
          10         20         30         40         50
 GATCCATCT AGAAGGAGGA ATAACATATG CCAGTAATGC ATCCTCATGG
BB      X
AI      B
MN      A
11      1

          60         70         80         90        100
TGCACCGCCA AATCATCGTC CGTGGCAAAT GAAAGATCTG CAGGCAATTA

         110        120        130        140        150
AGCAGGAAGT GTCTCAAGCA GCGCCGGGGT CACCTCAATT CATGCAGACC

         160        170        180        190        200
ATCCGCCTGG CCGTTCAACA GTTTGATCCG ACCGCTAAAG ACCTGCAAGA

         210        220        230
CCTGCTCCAG TATCTGTGCT CTAGCCTGGT TGCAAGCT
                                    HA
                                    IL
                                    NU
                                    31
```

The third oligonucleotide was initially replicated in plasmid M13mp19. Plasmid M13mp19 was purchased from New England BioLabs, Inc. Ligation was as described above. The construct was transfected into host cell JM103 (commercially available from many sources, including the ATCC). Transformants were plated

onto Luria Broth plates using soft agar containing untransfected JM103 cells, 5-bromo-4-chloro-3-indolyl-β-galactoside in dimethylformamide (1:120) and 333 μM isopropylthiogalactoside.

The plasmid M13mp19 was digested with XbaI and HindIII and the XbaI/HindIII fragment was isolated and then inserted into plasmid pADK1007 by digesting the plasmid with XbaI and HindIII and removing the existing XbaI/HindIII fragment and inserting the desired XbaI/HindIII fragment in its place. The new plasmid was designated pADK1026. The conditions to transform the host strain, FM6, with pADK1026 were similar to the conditions used with pADK1001, described above.

The directly expressed p24 gag polypeptide was purified as described in Example 10 or by high pressure liquid chromatography and the purified polypeptide was analyzed by protein microsequencing and amino acid composition. In addition, the antigenic polypeptide p24 gag was found to react with commercially available monoclonal antibodies (available from DuPont and Sigma) which are immunoreactive with virally derived antigenic proteins.

Example 8

Preparation of env B

The DNA sequence encoding the antigenic polypeptide, env B, was prepared by digesting the native env DNA sequence with SalI and BamHI to give the following SalI/BamHI fragment.

```
          10         20         30         40         50
TCGACGCTCC AGGATATGAC CCCATCTGGT TCCTTAATAC CGAACCCAGC
S
A
L
I

          60         70         80         90        100
CAACTGCCTC CCACCGCCCC TCCTCTACTC CCCCACTCTA ACCTAGACCA

         110        120        130        140        150
CATCCTCGAG CCCTCTATAC CATGGAAATC AAAACTCCTG ACCCTTGTCC

         160        170        180        190        200
AGTTAACCCT ACAAAGCACT AATTATACTT GCATTGTCTG TATCGATCGT

         210        220        230        240        250
GCCAGCCTCT CCACTTGGCA CGTCCTATAC TCTCCCAACG TCTCTGTTCC

         260        270        280        290        300
ATCCTCTTCT TCTACCCCCC TCCTTTACCC ATCGTTAGCG CTTCCAGCCC

         310        320        330        340        350
CCCACCTGAC GTTACCATTT AACTGGACCC ACTGCTTTGA CCCCCAGATT

         360        370        380        390        400
CAAGCTATAG TCTCCTCCCC CTGTCATAAC TCCCTCATCC TGCCCCCCTT

         410        420
TTCCTTGTCA CCTGTTCCCA CCCTAG GATC
                             B
                             A
                             M
                             H
                             I
```

The native HTLV-I DNA sequence used for the construction of env B was obtained from Irvin Chen at the University of California, Los Angeles, California. The DNA obtained from Chen was sequenced using the dideoxynucleotide technique of Sanger, (Proc. Natl. Acad. Sci., 74:5463 (1977)). A nucleotide change was noted at position 5997 of the HTLV-I sequence as described by Seiki, et al., Proc. Natl. Acad. Sci., 80:3618 (1983). This incorrect nucleotide caused premature termination of proteins attempted to be expressed using the sequence obtained from Chen. A synthetic oligomer with the sequence, 5'-CATTAACTGGACCCACTGC-3', was synthesized on an Applied BioSystems 380B DNA synthesizer and isolated by polyacrylamide gel electrophoresis. With the above oligomer, the incorrect nucleotide was thus changed to the correct nucleotide using a commercial site-directed mutagenesis kit (BioRad Cat. No. 170-3571). The mutated DNA was then sequenced again and found to be correct.

Initial attempts to express the mutated DNA sequence directly were unsuccessful and gave no detectible band having the appropriate molecular weight by Coomassie staining of SDS-polyacrylamide gels run on whole induced cells containing the mutated DNA sequence. Consequently, the mutated DNA sequence was modified by fusing it to a DNA sequence encoding the first 82 amino acids of alpha consensus interferon (described in U.S. Patent 4,695,623). This required the synthesis of a DNA duplex to insert as an in-frame coding sequence between the coding sequence for alpha consensus interferon (HindIII) and the coding sequence for this portion of the HTLV-I env protein (SalI). The sequence of the oligomer duplex was as follows:

5'AGCTTCGGTATGGGTTTCCCGTTCTCTCTGCTGG3'
3'AGCCATACCCAAAGGGCAAGAGAGACGACCAGCT5'

The linker oligomers were synthesized on an Applied BioSystems 380B DNA Synthesizer and the oligomers were annealed by mixing in a 1:1 ratio in sterile water, placing the mixture in boiling water for 5 minutes and allowing the solution to slowly cool to 4°C. The 5025 nucleotide vector DNA sequence containing the desired portion of alpha consensus interferon, as well as the E. coli expression vector pCFM1156, was isolated using NA-45 membrane (Schleicher & Schuell Applications Update No. 364). The 446 nucleotide insert SalI to BamHI DNA sequence encoding amino acids 158 through 308 of the HTLV-I env protein was isolated from the HTLV-I native sequence using NA-45 membrane (Schleicher and Schuell).

The three pieces of DNA were ligated together in a molar ratio of 1:3:3 (vector: insert: linker) following procedures described in Maniatis, et al., p. 126, transformed into E. coli strain FM6 as described above and selected for growth on Luria Broth agarose plates containing 50 ug/ml kanamycin at 28-30°C.

Viable clones were screened by restriction digestion. Clones which were determined to contain the correct insert were induced by growing the clones at 28°C in Luria Broth containing 50 ug/ml kanamycin until they reached an optical density at 600 nm of 0.3. The culture was then heated to 42°C and grown for an additional 5 hours.

The cells were harvested by spinning at 8000 rpm for 20 minutes in a Beckman J2-21 centrifuge. Harvested cells were lysed by microfluidization and the desired protein of the predicted molecular weight (26,500 daltons) was found in the inclusion body pellet when analyzed on SDS-PAGE.

The isolated env B antigenic polypeptide is expressed as a fusion polypeptide where the leader sequence is encoded by the following DNA sequence which expresses the amino acid sequence shown below.

```
          10         20         30         40         50
                                MetCys AspLeuProG lnThrHisSe
CTAGAAACCA TGAGGGTAAT AAATATGTGT GATTTACCTC AAACTCATTC
X
B
A
I

          60         70         80         90        100
rLeuGlyAsn ArgArgAlaL euIleLeuLe uAlaGlnMet ArgArgIleS
TCTTGGTAAC CGTCGCGCTC TGATTCTGCT GGCACAGATG CGTCGTATTT

         110        120        130        140        150
erProPheSe rCysLeuLys AspArgHisA spPheGlyPh eProGlnGlu
CCCCGTTTAG CTGCCTGAAA GACCGTCACG ACTTCGGCTT TCCGCAAGAA

         160        170        180        190        200
GluPheAspG lyAsnGlnPh eGlnLysAla GlnAlaIleS erValLeuHi
GAGTTCGATG GCAACCAATT CCAGAAAGCT CAGGCAATCT CTGTACTGCA

         210        220        230        240        250
sGluMetIle GlnGlnThrP heAsnLeuPh eSerThrLys AspSerSerA
CGAAATGATC CAACAGACCT TCAACCTGTT TTCCACTAAA GACAGCTCTG

         260        270
laAlaTrpAs pGlu
CTGCTTGGGA CGAAAGCT
             H
             I
             N
             3
```

The leader amino acid sequence may be cleaved from the antigenic polypeptide sequence using CNBr because the desired antigenic polypeptide amino acid sequence contains no internal methionines.

Example 9

Preparation of $p40^X$

The native HTLV-I sequence used for the construction of expression of $p40^X$ was obtained from Irvin Chen at the University of California, Los Angeles, California. The vector containing the $p40^X$ coding sequence was digested with AccI and EcoRI and the 2.9 kb fragment containing the desired portion of the tax gene was isolated using agarose gel purification with NA-45 membrane paper (Schleicher and Schuell). The expression vector pCFM1156 was cleaved with XbaI and EcoRI and also purified using agarose gel electrophoresis and NA-45 membrane paper (Schleicher and Schuell). A synthetic DNA linker with the following sequence was synthesized:

```
        10         20         30         40         50
CTAGAAGGAG GAATAACATA TGGCACATTT TCCGGGTTTC GGCCAGTCTC
    TTCCTC CTTATTGTAT ACCGTGTAAA AGGCCCAAAG CCGGTCAGAG
X
B
A
I

        60         70
TGCTGTTCGG TTACCCGGT
ACGACAAGCC AATGGGCCAGA
                   A
                   C
                   C
                   I
```

The linker was kinased and ligated onto the purified AccI/EcoRI fragment of the native HTLV-I tax DNA sequence. The resulting composite synthetic/native tax gene was isolated using agarose gel electrophoresis and NA-45 membrane paper (Schleicher and Schuell). This XbaI/EcoRI DNA fragment was ligated into pCFM1156 cleaved as described above. No expression was observed of any candidate clones. Upon dideoxynucleotide DNA sequencing of one of the clones, nucleotides near the XbaI site were found to be deleted.

Therefore, a synthetic DNA sequence from the ClaI site of pCFM1156 to the ClaI site of the native $p40^{x}$ sequence was synthesized to reinsert the deleted nucleotides and to afford the advantages of a nucleotide sequence optimized for expression in E.coli.

The following synthetic DNA sequence was prepared using the known procedures described above.

```
        10         20         30         40         50
  CGATTTGA TTCTAGAAGG AGGAATAACA TATGGCACAT TTTCCGGGTT
    TAAACT AAGATCTTCC TCCTTATTGT ATACCGTGTA AAAGGCCCAA
C
L
A
I

        60         70         80         90        100
TCGGCCAAAG CTTACTGTTC GGTTACCCGG TATACGTATT CGGCGACTGC
AGCCGGTTTC GAATGACAAG CCAATTGGGCC ATATGCATAA GCCGCTGACG

       110        120        130        140        150
GTTCAGGGTG ATTGGTGCCC TATCTCTGGT GGCCTGTGTT CCGCTCGTCT
CAAGTCCCAC TAACCACGGC ATAGAGACCA CCGGACACAA GGCGAGCAGA
```

```
        160         170         180         190         200
GCACCGCCAT GCACTGCTGG CGACTTGCCC GGAACACCAA ATTACCTGGG
CGTGGCGGTA CGTGACGACC GCTGAACGGG CCTTGTGGTT TAATGGACCC

        210
ACCCGAT
TGGGCTAGC
     C
     L
     A
     I
```

The synthetic sequence contains a ClaI restriction site for ligation to the ClaI site of the plasmid, pCFM1156, and encodes the native amino acid sequence for the $p40^{x}$ polypeptide up to ClaI site of the native $p40^{x}$ gene. The synthetic sequence was designed to use the optimal codon usage for E. coli and minimized undesired secondary mRNA structure. The expression vector containing the modified XbaI site was cleaved with ClaI and agarose gel purified. The synthetic ClaI/ClaI DNA sequence was ligated into the ClaI-cleaved vector. Transformants were screened for correct orientation of the inserted synthetic DNA fragment and those with the fragment in the correct orientation were induced as above. A polypeptide band of the predicted molecular weight was seen in the inclusion body fraction of induced cells containing the proper construct when analyzed on SDS-PAGE.

The desired recombinant polypeptide, $p40^{x}$, was immunologically identified by a Western blot using antiserum generated to a peptide containing amino acids 269 to 353 of the carboxy terminus of the native HTLV-I tax protein developed jointly by Amgen and Dennis Slamon at the University of California, Los Angeles, California.

The recombinant polypeptide was negative by Western blot using normal rabbit serum. In addition, the recombinant $p40^{x}$ polypeptide was identified immunologically by Western blot using HTLV-I positive human serum (obtained from Stan Weiss at the University of New Jersey Medical School). The recombinant protein was negative by Western blot using normal human serum.

Example 10

Purification of the p24 gag Polypeptide

The crude polypeptide prepared as described in Example 7 is diluted to 50 ml with 4 M Guanidine HCl: 100 mM DTT:50 mM Tris-HCl, pH 8.0, stirred briefly, and transferred into a pre-washed Spectra/Por 1, 6-8K molecular weight cutoff dialysis tubing (51mm). The solution is dialyzed against 4 liters of 50 mM Tris-HCl pH 8.0:1 mM DTT twice (6 h and 12 h), then 4 liters of 10 mM Tris-HCl, pH 8.0:1 mM DTT twice (6 h and 18 h). Dialyzed material is centrifuged at 8K for 10 minutes. The supernatant is decanted and loaded onto an open column of CM-52 (75 ml bed volume), which had been packed in 100 mM Tris HCl pH 8.0 and washed with 300 ml of 10 mM HCl, pH 8.0:1 mM DTT then eluted with 175 ml of 50 mM NaCl: 10 mM Tris: 1 mM DTT. This elution pool is lyophilized, resolubilized in 100 ml of 2 M Guanidine HCl:50 mM Tris HCl pH 8.0:100 mM DTT, dialyzed in Spectra/Por, 1 6-8K molecular weight cutoff tubing against 4 liters of water (twice), 4 liters of 0.1% TFA (twice), and lyophilized.

Example 11

Purification of the $p40^x$ Polypeptide

The crude polypeptide prepared as described in Example 9 is added to 10 ml of 8 M Guanidine HCl:100 mM DTT:50 mM Tris-HCl, pH 8.0 and stirred for 2 hours. The mixture is diluted to 2.5 M Guanidine HCl with 22 ml of 50 mM Tris-HCl, pH 8.0, and centrifuged at 5K for 5 minutes. The supernatant is decanted away and the pellet is solubilized in 30 ml of 45% aqueous CH_3CN:55% 0.1% TFA with sonication (high power, 2 to 3 minutes). The mixture is centrifuged at 15K for 10 minutes, filtered through a 0.45 micron filter, and chromatographed. A first purification is performed on a Vydac WPC_4 semi-prep column (1.0 x 25 cm) using a linear gradient of 40-60% (0.05% TFA in CH_3CN, 0.1% aqueous TFA) over 20 minutes. The proper fractions are combined and lyophilized. The dry material is resolubilized in 15 ml of 45% CH_3CN:55% 0.1% aqueous TFA and reprepped on a Vydac WPC_{18} semi-prep column (1.0 x 25cm) using a linear gradient of 45-55% over 20 minutes (0.05% TFA in CH_3CN, 0.1% aqueous TFA). The proper fractions are collected and lyophilized.

Example 12

Purification of env Polypeptides

The crude polypeptide prepared as described in Example 8 is suspended in 20 ml of water and centrifuged at 15K for 10 minutes. The supernatant is decanted away and the pellet solubilized in 10 ml 8 M Guanidine HCl: 100 mM DTT:50 mM Tris-HCl, pH 8.0, with stirring for 2 hours. The solution is diluted to 2 M Guanidine HCl with 30 ml of 50 mM Tris-HCl, pH 8.0, with formation of a precipitate which is pelletized by centrifugation at 5K for 5 minutes. The supernatant is decanted away and

the pellet is solubilized immediately in 40 ml of 45% CH_3CN:55% 0.1% aqueous TFA with sonication (high power, 2-3 minutes). The solution is centrifuged at 15K for 10 minutes, filtered through 0.45 micron filter and HPLC purified on Vydac WPC_{18} semi-prep column (1.0 X 25cm) using a linear gradient of 35-60% over 25 minutes (0.05% TFA in CH_3CN, and 0.1% aqueous TFA). The proper fractions are combined and lyophilized.

While the present invention has been described in terms of specific methods and compositions, it is understood that variations and modifications will occur to those skilled in the art upon consideration of the above description and examples. Accordingly, the appended claims are intended to cover all equivalent variations of the present invention.

CLAIMS

1. A method for detecting exposure to HTLV-I comprising:

combining a body fluid containing an antibody with an amount of one or more recombinant polypeptide antigens selected from the group consisting of polypeptides encoded by all or part of the env, tax, and gag genes of HTLV-I effective to maximize the sensitivity and selectivity of an immunological assay, wherein said polypeptides are bound to a solid support;

forming a detectable antibody-antigen binding pair between said antibody and polypeptide; and

detecting the presence of said pair on said support.

2. The method of claim 1 wherein said support is a microtiter well.

3. The method of claim 2 wherein said polypeptides are env B, $p40^x$ or p24 gag.

4. The method of claim 3 wherein said support has about 1.89×10^{-13} to 3.78×10^{-11} moles env B, 1.25×10^{-13} to 2.50×10^{-11} moles $p40^x$, or 2.09×10^{-13} to 8.34×10^{-11} moles p24 gag bound thereto.

5. The method of claim 4 wherein said support has about 3.78×10^{-13} to 9.45×10^{-12} moles env B, 2.5×10^{-13} to 6.25×10^{-12} moles p 40^x, or 4.17×10^{-13} to 2.08×10^{-11} moles of p24 gag bound thereto.

6. The method of claim 5 wherein said support has about 1.89×10^{-12} moles env B, 1.25×10^{-12} moles $p40^x$, or 1.04×10^{-11} moles of p24 gag bound thereto.

7. A method for detecting exposure to HTLV-I comprising:

combining a body fluid containing an antibody with an amount of at least three recombinant polypeptide antigens selected from the group consisting of polypeptides encoded by all or part of the env, tax, and gag genes of HTLV-I, effective to maximize the sensitivity and selectivity of an immunological assay, wherein said polypeptides are bound to a solid support;

forming a detectable antibody-antigen binding pair between said antibody and polypeptide; and

detecting the presence of said pair on said support.

8. The method of claim 7 wherein said support is a microtiter well.

9. The method of claim 7 wherein said polypeptides are env B, $p40^x$, and p24 gag.

10. The method of claim 9 wherein said support has about 1.89×10^{-13} to 3.78×10^{-11} moles env B, 1.25×10^{-13} to 2.50×10^{-11} moles $p40^x$, and 2.09×10^{-13} to 8.34×10^{-11} moles p24 gag bound thereto.

11. The method of claim 10 wherein said support has about 3.78×10^{-13} to 9.45×10^{-12} moles env B, 2.5×10^{-13} to 6.25×10^{-12} moles $p40^x$, and 4.17×10^{-13} to 2.08×10^{-11} moles of p24 gag bound thereto.

12. The method of claim 11 wherein said support has about 1.89×10^{-12} moles env B, 1.25×10^{-12} moles $p40^x$, and 1.04×10^{-11} moles of p24 gag bound thereto.

13. The method of claim 7 wherein said solid support is nitrocellulose.

14. An assay kit for detecting a HTLV-I antibody comprising:

a) a solid support coated with an amount of at least three recombinant polypeptide antigens selected from the group consisting of polypeptides encoded by all or part of the env, tax and gag genes of HTLV-I effective to maximize the sensitivity and selectivity of an immunological assay, wherein binding sites on said support not containing said polypeptides are blocked;

b) antigen-antibody binding pair detection reagents; and

c) developing reagents.

15. The kit of claim 14 wherein said solid support comprises a microtiter well having env B, $p40^x$, and p24 gag bound thereto.

16. The kit of claim 15 wherein said well has about 1.89×10^{-13} to 3.78×10^{-11} moles env B, 1.25×10^{-13} to 2.5×10^{-11} moles $p40^x$, and 2.09×10^{-13} to 8.34×10^{-11} moles p24 gag bound thereto.

17. The kit of claim 16 wherein said well has about 3.78×10^{-13} to 9.45×10^{-12} moles env B, 2.5×10^{-13} to 6.25×10^{-12} moles $p40^x$, and 4.17×10^{-13} to 2.08×10^{-11} moles p24 gag bound thereto.

18. The kit of claim 17 wherein said well has about 1.89×10^{-12} moles env B, 1.25×10^{-12} moles $p40^x$, and 1.04×10^{-11} moles p24 gag bound thereto.

19. The kit of claim 14 wherein said detection reagents comprise labelled goat antihuman antibody.

20. A competition immunoassay to detect the presence of an antibody to an HTLV-I antigen comprising:

a) preparing identical first and second dilutions of a body fluid containing an antibody;

b) generating a detectable signal using said first dilution and a recombinant polypeptide antigen, wherein said HTLV-I antibody forms an antigen-antibody binding pair, and said polypeptide antigen is selected from the group consisting of purified env B, $p40^X$, and p24 gag polypeptides;

c) adding a known amount of a recombinant polypeptide antigen selected from the group consisting of purified env B, $p40^X$, and p24 gag polypeptides to said second dilution, wherein said antigen is the antigen of b);

d) generating a detectable signal using said second dilution, wherein said HTLV antibody forms a detectable antigen-antibody binding pair with said antigen of b); and

e) determining the presence of said HTLV antibody in said first dilution by comparing the difference of said signal of b) and said signal of d).

21. An immunological composition for use in assays to detect a HTLV-I antibody comprising:

a) a solid support;

b) an amount of at least three purified recombinant polypeptide antigens selected from the group consisting of polypeptides encoded by all or part of the env, tax, and gag genes of HTLV-I effective to maximize the sensitivity and selectivity of an immunological assay; and

c) an amount of a blocking agent bound to said support sufficient to minimize nonspecific binding.

22. The DNA sequence of Figure 1 or fragments thereof encoding the p24 gag polypeptide.

23. The DNA sequence of Figure 2 or fragments thereof encoding the fusion polypeptide env B.

24. The DNA sequence of Figure 3 or fragments thereof encoding $p40^{x}$ polypeptide.

25. The antigenic fusion polypeptide env B.

FIGURE 1A

DNA AND AMINO ACID SEQUENCE OF ENV B

```
        10          20          30          40          50          60          70          80
                                METCYS ASPLEUPROG LNTHRHISSE RLEUGLYASN ARGARGALAL EUILELEULE
CTAGAAACCA TGAGGGTAAT AAATATGTGT GATTTACCTC AAACTCATTC TCTTGGTAAC CGTCGCGCTC TGATTCTGCT
X
B
A
I

        90          100         110         120         130         140         150         160
UALAGLNMET ARGARGILES ERPROPHESE RCYSLEULYS ASPARGHISA SPPHEGLYPH EPROGLNGLU GLUPHEASPGL
GGCACAGATG CGTCGTATTT CCCCGTTTAG CTGCCTGAAA GACCGTCACG ACTTCGGCTT TCCGCAAGAA GAGTTCGATGG

        170         180         190         200         210         220         230         240
YASNGLNPHE GLNLYSALAG LNALAILESE RVALLEUHIS GLUMETILEG LNGLNTHRPH EASNLEUPHE SERTHRLYSA
CAACCAATTC CAGAAAGCTC AGGCAATCTC TGTACTGCAC GAAATGATCC AACAGACCTT CAACCTGTTT TCCACTAAAG
```

FIGURE 1B

```
       250        260        270        280        290        300        310        320
SPSERSERAL AALATRPASP GLUSERPHEG LYMETGLYPH EPROPHESER LEULEUVALA SPALAPROGL YTYRASPPRO
ACAGCTCTGC TGCTTGGGAC GAAAGCTTCG GTATGGGTTT CCCGTTCTCT CTGCTGGTCG ACGCTCCAGG ATATGACCCC
                      H                             S
                      I                             A
                      N                             L
                      3                             I

       330        340        350        360        370        380        390        400
ILETRPPHEL EUASNTHRGL UPROSERGLN LEUPROPROT HRALAPROPR OLEULEUPRO HISSERASNL EUASPHISIL
ATCTGGTTCC TTAATACCGA ACCCAGCCAA CTGCCTCCCA CCGCCCCTCC TCTACTCCCC CACTCTAACC TAGACCACAT

       410        420        430        440        450        460        470        480
ELEUGLUPRO SERILEPROT RPLYSSERLY SLEULEUTHR LEUVALGLNL EUTHRLEUGL NSERTHRASN TYRTHRCYSI
CCTCGAGCCC TCTATACCAT GGAAATCAAA ACTCCTGACC CTTGTCCAGT TAACCCTACA AAGCACTAAT TATACTTGCA

       490        500        510        520        530        540        550        560
LEVALCYSIL EASPARGALA SERLEUSERT HRTRPHISVA LLEUTYRSER PROASNVALS ERVALPROSE RSERSERSER
TTGTCTGTAT CGATCGTGCC AGCCTCTCCA CTTGGCACGT CCTATACTCT CCCAACGTCT CTGTTCCATC CTCTTCTTCT

       570        580        590        600        610        620        630        640
THRPROLEUL EUTYRPROSE RLEUALALEU PROALAPROH ISLEUTHRLE UPROPHEASN TRPTHRHISC YSPHEASPPR
ACCCCCCTCC TTTACCCATC GTTAGCGCTT CCAGCCCCCC ACCTGACGTT ACCATTTAAC GTGACCCACT GCTTTGACCC
```

FIGURE 1C

```
       650        660        670        680        690        700        710        720
OGLNILEGLN ALAILEVALS ERSERPROCY SHISASNSER LEUILELEUP ROPROPHESE RLEUSERPRO VALPROTHRL
CCAGATTCAA GCTATAGTCT CCTCCCCCTG TCATAACTCC CTCATCCTGC CCCCCTTTTC CTTGTCACCT GTTCCCACCC

       730
EU
TA
```

FIGURE 2A

DNA AND AMINO ACID SEQUENCE OF P24 GAG

```
        10         20         30         40         50         60         70         80
                              MET PROVALMETH ISPROHISGL YALAPROPRO ASNHISARGP ROTRPGLNME
GGATCCATCT AGAAGGAGGA ATAACATATG CCAGTAATGC ATCCTCATGG TGCACCGCCA AATCATCGTC CGTGGCAAAT
BB      X
AI      B
MN      A
II      I

        90         100        110        120        130        140        150        160
TLYSASPLEU GLNALAILEL YSGLNGLUVA LSERGLNALA ALAPROGLYS ERPROGLNPH EMETGLNTHR ILEARGLEUA
GAAAGATCTG CAGGCAATTA AGCAGGAAGT GTCTCAAGCA GCGCCGGGGT CACCTCAATT CATGCAGACC ATCCGCCTGG

        170        180        190        200        210        220        230        240
LAVALGLNGL NPHEASPPRO THRALALYSA SPLEUGLNAS PLEULEUGLN TYRLEUCYSS ERSERLEUVA LALASERLEU
CCGTTCAACA GTTTGATCCG ACCGCTAAAG ACCTGCAAGA CCTGCTCCAG TATCTGTGCT CTAGCCTGGT TGCAAGCTTG
                                                                  H
                                                                  L
                                                                  N
                                                                  3
```

FIGURE 2B

```
       250        260        270        280        290        300        310        320
HISHISGLNG LNLEUASPSE RLEUILESER GLUALAGLUT HRARGGLYIL ETHRGLYTYR ASNPROLEUA LAGLYPROLE
CACCATCAAC AGCTGGACAG CCTGATTTCC GAAGCGGAAA CACGCGGTAT CACCGGCTAC AACCCGCTGG CGGGTCCACT

       330        340        350        360        370        380        390        400
UARGVALGLN ALAASNASNP ROGLNGLNGL NGLYLEUARG ARGGLUTYRG LNGLNLEUTR PLEUALAALA PHEALAALAL
GCGTGTTCAA GCCAACAATC CTCAGCAACA GGGTCTGCGT CGCGAATATC AACAGCTGTG GCTCGCGGCA TTCGCTGCGC

       410        420        430        440        450        460        470        480
EUPROGLYSE RALALYSASP PROSERTRPA LASERILELE UGLNGLYLEU GLUGLUPROT YRHISALAPH EVALGLUARG
TGCCGGGCTC TGCGAAAGAT CCGAGCTGGG CTTCTATTCT GCAAGGTCTC GAGGAACCGT ACCACGCCTT CGTGGAACGT

       490        500        510        520        530        540        550        560
LEUASNILEA LALEUASPAS NGLYLEUPRO GLUGLYTHRP ROLYSASPPR OILELEUARG SERLEUALAT YRSERASNAL
CTGAACATCG CTCTGGATAA TGGCCTGCCG GAAGGTACCC CGAAAGATCC TATTCTGCGC AGCCTGGCGT ACAGCAACGC

       570        580        590        600        610        620        630        640
AASNLYSGLU CYSGLNLYSL EULEUGLNAL AARGGLYHIS THRASNSERP ROLEUGLYAS PMETLEUARG ALACYSGLNT
GAACAAAGAA TGTCAAAAAC TGCTGCAAGC TCGTGGTCAC ACAAATAGCC CGCTGGGCGA TATGCTGCGT GCATGCCAAA

       650        660        670
HRTRPTHRPR OLYSASPLYS THRLYSVALL EUEND
CCTGGACTCC GAAAGATAAG ACCAAAGTGC TGTAA
```

FIGURE 3A

DNA AND AMINO ACID SEQUENCE OF $P40^{x}$

```
        10         20         30         40         50         60         70         80
ATGGCACATT TTCCGGGTTT CGGCCAAAGC TTACTGTTCG GTTACCCGGT ATACGTATTC GGCGACTGCG TTCAGGGTGA
METALAHISP HEPROGLYPH EGLYSLNSER LEULEUPHEG LYTYRPROVA LTYRVALPHE GLYASPCYSV ALGLNGLYAS

        90        100        110        120        130        140        150        160
TTGGTGCCCT ATCTCTGGTC GCCTGTGTTC CGCTCGTCTG CACCGCCATG CACTGCTGGC GACTTGCCCG GAACACCAAA
PTRPCYSPRO ILESERGLYG LYLEUCYSSE RALAARGLEU HISARGHISA LALEULEUAL ATHRCYSPRO GLUHISGLNI

       170        180        190        200        210        220        230        240
TTACCTGGGA CCCGATCGAT GGACGCGTTA TCGGCTCAGC TCTACAGTTC CTTATCCCTC GACTCCCCTC CTTCCCCACC
LETHRTRPAS PPROILEASP GLYARGVALI LEGLYSERAL ALEUGLNPHE LEUILEPROA RGLEUPROSE RPHEPROTHR

       250        260        270        280        290        300        310        320
CAGAGAACCT CTAAGACCCT CAAGGTCCTT ACCCCGCCAA TCACTCATAC AACCCCCAAC ATTCCACCCT CCTTCCTCCA
GLNARGTHRS ERLYSTHRLE ULYSVALLEU THRPROPROI LETHRHISTH RTHRPROASN ILEPROPROS ERPHELEUGL

       330        340        350        360        370        380        390        400
GGCCATGCGC AAATACTCCC CCTTCCGAAA TGGATACATG GAACCCACCC TTGGGCAGCA CCTCCCAACC CTGTCTTTTC
NALAMETARG LYSTYRSERP ROPHEARGAS NGLYTYRMET GLUPROTHRL EUGLYGLNHI SLEUPROTHR LEUSERPHEP
```

FIGURE 3B

```
       410        420        430        440        450        460        470        480
CAGACCCCGG ACTCCGGCCC CAAAACCTGT ACACCCTCTG GGGAGGCTCC GTTGTCTGCA TGTACCTCTA CCAGCTTTCC
ROASPPROGL YLEUARGPRO GLNASNLEUT YRTHRLEUTR PGLYGLYSER VALVALCYSM ETTYRLEUTY RGLNLEUSER

       490        500        510        520        530        540        550        560
CCCCCCATCA CCTGGCCCCT CCTGCCCCAC GTGATTTTTT GCCACCCCGG CCAGCTCGGG GCCTTCCTCA CCAATGTTCC
PROPROILET HRTRPPROLE ULEUPROHIS VALILEPHEC YSHISPROGL YGLNLEUGLY ALAPHELEUT HRASNVALPR

       570        580        590        600        610        620        630        640
CTACAAGCGA ATAGAAGAAC TCCTCTATAA AATTTCCCTC ACCACAGGGG CCCTAATAAT TCTACCCGAA GACTGTTTGC
OTYRLYSARG ILEGLUGLUL EULEUTYRLY SILESERLEU THRTHRGLYA LALEUILEIL ELEUPROGLU ASPCYSLEUP

       650        660        670        680        690        700        710        720
CCACCACCCT TTTCCAGCCT GCTAGGGCAC CCGTCACGCT AACAGCCTGG CAAAACGGCC TCCTTCCGTT CCACTCAACC
ROTHRTHRLE UPHEGLNPRO ALAARGALAP ROVALTHRLE UTHRALATRP GLNASNGLYL EULEUPROPH EHISSERTHR

       730        740        750        760        770        780        790        800
CTCACCACTC CAGGCCTTAT TTGGACATTT ACCGATGGCA CGCCTATGAT TTCCGGGCCC TGCCCTAAAG ATGGCCAGCC
LEUTHRTHRP ROGLYLEUIL ETRPTHRPHE THRASPGLYT HRPROMETIL ESERGLYPRO CYSPROLYSA SPGLYGLNPR

       810        820        830        840        850        860        870        880
ATCTTTAGTA CTACAGTCCT CCTCCTTTAT ATTTCACAAA TTTCAAACCA AGGCCTACCA CCCCTCATTT CTACTCTCAC
OSERLEUVAL LEUGLNSERS ERSERPHEIL EPHEHISLYS PHEGLNTHRL YSALATYRHI SPROSERPHE LEULEUSERH
```

FIGURE 3C

```
       890        900        910        920        930        940        950        960
ACGGCCTCAT ACAGTACTCT TCCTTTCATA GTTTACATCT CCTGTTTGAA GAATACACCA ACATCCCCAT TTCTCTACTT
ISGLYLEUIL EGLNTYRSER SERPHEHISS ERLEUHISLE ULEUPHEGLU GLUTYRTHRA SNILEPROIL ESERLEULEU

       970        980        990       1000       1010       1020       1030       1040
TTTAACGAAA AAGAGGCAGA TGACAATGAC CATGAGCCCC AAATATCCCC CGGGGGCTTA GAGCCTCCCA GTGAAAAACA
PHEASNGLUL YSGLUALAAS PASPASNASP HISGLUPROG LNILESERPR OGLYGLYLEU GLUPROPROS ERGLULYSHI

      1050       1060
TTTCCGAGAA ACAGAAGTCT GA
SPHEARGGLU THRGLUVALE ND
```

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/06647

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C12Q 1/70
U.S. CL.: 435/5

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S. Cl.:	435/5

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	US, A, 06/664972 (Papas et al.) 26 October 1984. See entire document.	1-25
Y	EP, A, 0,136,798 (Bodner et al.) 21 September 1984. see entire document.	1-25
Y	Molecular and Cellular Probes, Volume 2. Issued 1988, Kitajima et al., "Synthesis of proteins in Escherichia coli immunoreactive with sera from individuals infected with human T-cell leukemia virus type 1", pages 39-46, see entire document.	1-25

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
26 December 1990	22FEB 1991
International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	for Bradley L. Sisson

Form PCT/ISA/210 (second sheet) (May 1986)